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TEMPORAL ABUNDANCE, PARITY, SURVIVAL RATES, AND ARBOVIRUS ISOLATION OF FIELD-COLLECTED CONTAINER-INHABITING MOSQUITOES IN EASTERN TENNESSEE

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ABSTRACT. Surveillance of container-inhabiting mosquitoes was conducted from June 17 through November 9, 1998, at 2 1997 La Crosse virus (LAC) human case sites (Knox and Cocke counties, Tennessee). Mosquitoes were collected weekly with 2 dry ice–baited Centers for Disease Control miniature light traps, 2 omnidirectional Fay traps, and 40 oviposition traps at each site. A total of 8,408 mosquitoes, composed of *Ochlerotatus triseriatus* (n = 2,095) and *Aedes albopictus* (n = 6,313), were reared or collected and assayed for virus. The majority of host-seeking *Ae. albopictus* (n = 567) collected from July through October from both sites were dissected to determine parity status. Monthly parity rates ranged from 0.78 to 0.85 and 0.79 to 0.92 in Knox and Cocke counties, respectively. The high parity rates indicate that this population of *Ae. albopictus* has a high daily survival rate and may have a high vector potential. The temporal patterns in *Ae. albopictus* and *Oc. triseriatus* egg collections from both of the human case sites were significantly correlated, suggesting that the populations fluctuate in a similar manner across the eastern Tennessee region. Although LAC was not isolated from either species, one isolation of a California serogroup virus, most likely a subtype of Jamestown Canyon virus (JC), was recovered from a pool of 50 male *Ae. albopictus* reared from eggs collected at the Knox County site (minimum field infection rate of 1.89 per 1,000). This is the 1st report of a very closely related JC-like virus in *Ae. albopictus* and from Tennessee, as well as the 1st time this potential human pathogen has been isolated from transovarially infected field populations of *Ae. albopictus.*

KEY WORDS *Ochlerotatus triseriatus, Aedes albopictus,* parity, temporal abundance, Jamestown Canyon virus, California serogroup

INTRODUCTION

From 1963 to 1996, 9 cases of pediatric La Crosse viral encephalitis were reported to the Tennessee Department of Health. Since 1997, confirmed cases have increased to approximately 9–10 per year in the eastern region of Tennessee (Jones et al. 1999). These recent health developments led to a surveillance program for container-inhabiting mosquitoes at selected human case sites. Information about the biology of *Ochlerotatus triseriatus* (Say) and *Aedes albopictus* (Skuse) in the continental southeastern United States is necessary to determine indicators of potential epidemiologic importance of endemic arbovirus cycles (Willis and Nasci 1994). The density of mosquito populations is not the only factor that determines vector potential and influences vector biology. Other important factors include survivorship, parity rates, blood-feeding patterns, and ecological factors such as nutritional quality of larval habitats, relative availability of hosts, and weather.

Jamestown Canyon virus (JC; *Bunyaviridae*: *Bunyavirus*) is a member of the California virus (CAL) serogroup of arboviruses and has been isolated from more than 20 mosquito species and tabanid flies. Jamestown Canyon virus is enzootic throughout temperate North America (Grimstad 1983) and has the widest North American distribution of the CAL serogroup viruses (Grimstad 1988).

Aedes albopictus was recently introduced into the continental United States and has spread rapidly to many areas (Moore and Mitchell 1997), including nearly every county in Tennessee (Moore 1998). *Aedes albopictus* and *Oc. triseriatus* were the most commonly collected mosquitoes during a 1998–99 mosquito surveillance program in 13 eastern Tennessee counties (Gottfried 1999). Additionally, *Ae. albopictus* is responsible for the vast majority of mosquito complaints received by the Tennessee Agricultural Extension Service (K. Vail, personal communication).

The objective of this study was to examine the container-inhabiting mosquitoes collected from the vicinity of human La Crosse viral encephalitis case sites to determine temporal patterns, parity, survival rates, and arbovirus activity.

MATERIALS AND METHODS

Collection sites: Eggs and adults of *Ae. albopictus* and *Oc. triseriatus* were collected from 1 site each in Knox and Cocke counties, Tennessee (June 17–November 9, 1998). Both sites were the residences of children who had contracted confirmed cases of La Crosse viral encephalitis during the summer of 1997. The Knox County residence (35°59′N, 84°08′W) is located in the Karns com-

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munity near the northwestern boundary of the county. This middle-income community consists of homes built in clearings in a mixed hardwood forest. Several multihectare blocks of forest are in close proximity to the index site. The primary overstory species are tulip poplar (*Liriodendron tulipifera*), black oak (*Quercus velutina*), Virginia pine (*Pinus virginiana*), and white oak (*Quercus alba*). Understory species include red maple (*Acer rubum*), eastern redbud (*Cercis canadensis*), red elm (*Ulmus rubra*), and Virginia creeper (*Parthenocissus quinquefolia*). Although the homes and yards of the community were well maintained (no trash dumps or discarded tires), artificial containers (children's toys, buckets, and birdbaths) were present that could support mosquito production. One sampling site in a forested area 300 m from the index site contained roadside discards of bottles, cans, and other containers.

The Cocke County residence (35°52′N, 83°13′W) is located in the community of Cosby, TN, and is bordered on the west by Middle Creek. This rural residence is a house trailer with numerous artificial containers littering the yard, located within 25 m of a private campground. The overstory species are principally hackberry (*Celtis occidentalis*) and boxelder (*Acer negundo*). Understory species are tall grasses and bamboo. A mature oak–hickory forest with an overstory of northern red oak (*Quercus rubra*), American beech (*Fagus grandifolia*), and hickory (*Carya* spp.) occurs within 100 m of the home. Understory species are red maple and American beech.

Ovitrapping: Eggs of *Ae. albopictus* and *Oc. triseriatus* were collected weekly from both sites with oviposition traps. Each site had 4 sets of 10 473-ml black (inside and outside) plastic cups attached to the north side of trees approximately 0.5 m from the base and spaced approximately 10 m apart (Loor and DeFoliart 1969). Two holes were cut below the rim of the cup with a paper hole punch for drainage and the cups were filled with 400 ml of tap water. Paper strips, 5 × 25.5 cm of 76-lb seed germination paper (Anchor Paper Company, Saint Paul, MN), were attached to the inside of the cups with paper clips to serve as the oviposition substrates (Steinly et al. 1991). Each week, the oviposition traps were replenished by replacing the oviposition strips and supplying fresh tap water. The strips from the previous week were placed in labeled plastic bags and transported to the laboratory for species identification and egg counting.

Host-seeking adult collections: Host-seeking mosquitoes were collected weekly from both sites with 2 dry ice–baited Centers for Disease Control miniature light traps (light removed) and 2 dry ice–baited omnidirectional Fay traps. The dry ice was contained in a 5.68-liter water cooler suspended adjacent to the trap, and the nozzle of the cooler dispenser was open to allow CO_2 to escape in the trap vicinity. Two traps of each type were deployed in the immediate area of the residence for a 24-h period at both sites.

Rearing of ovitrap specimens: The oviposition strips were air dried in the laboratory and eggs were identified, counted by species (Pratt and Kidwell 1969), and stored until rearing. The eggs were reared to the adult stage by submerging groups of 10 strips from each site by week in 2.0 liters of tap water with 2.5 g of dried liver powder per liter in a white plastic tray. Larvae were maintained at 27 ± 2°C until a majority reached the pupal stage. At that point, all developmental stages were placed into Mosquito Breeders (BioQuip, Gardena, CA). Adults that emerged were killed by freezing and immediately sorted by species and sex. Sorted mosquitoes were placed into lots of not more than 50 individuals/pool and stored at −70°C until tested for the presence of virus.

Rearing success: Rearing success was calculated for each species by site and was expressed as the number of adults (male and female) reared from eggs divided by the total number of eggs collected times 100. The viability of the eggs was evaluated by bleaching the pigmented chorion (Trpis 1970) and visualizing the immature larvae. Oviposition strips were immersed in a bleaching solution (3 g of sodium chlorite, 2 ml of glacial acetic acid, and 1,000 ml of distilled water) for 12 h. The tissue contents of the egg could be seen through the bleached chorion under a compound microscope and viable immature larvae could be distinguished from desiccated tissue.

Virus isolation and identification: The mosquito pools were homogenized in a diluent and the supernatant was inoculated on Vero cells to detect the presence of virus (Gerhardt et al. 2001). The positive original mosquito homogenate was reinoculated on Vero cells to confirm the presence of virus. The minimum field infection rate (MIR) was expressed as the number of pools positive for virus over the total number of specimens of that species tested during the sampling period (Nasci and Mitchell 1996).

Viral isolate TN98-5085 was tested by 1-way neutralization tests (Lindsey et al. 1976) against single-injection antibody to 7 domestic CAL serogroup viruses. Homologous plaque-reduction neutralization test (PRNT) titers were predetermined for the 7 sera and included antibody to California encephalitis virus, JC, Keystone virus (KEY), La Crosse virus (LAC), San Angelo virus, snowshoe hare virus, and trivittatus virus and a type-specific neutralizing monoclonal antibody (MCA) to JC (Artsob et al. 1992). Homologous PRNT titers of the JC MCA were 1,280–2,560 for prototype JC and varied from <10 to 2,560 for other JC-like isolates.

The virus isolate was also characterized by reverse transcriptase polymerase chain reaction (RT-PCR) and nucleic acid (na) sequence analysis. Viral RNA was extracted from infected Vero cell culture super-

Table 1. Jamestown Canyon virus M-segment primers.

Primer	Sequence
JCM7F	TAC TAC CAA GTA TAG AAA ACG TTC AA
JCM821F	GGC TTA GTT TAT CAC CCA TTC A
JCM1093F	TGA TAT GGT ACA AAT GGC GGA GAG
JCM1544F	CTA AAT TTA GGT AGA TGC GAC AA
JCM2001F	AAG CAT TTA GAG GCA CTG GA
JCM468R	GTC CCA GCA ACC TCA AAG TG
JCM845R	CTG TGA ATG GGT GAT AAA CTA AGC
JCM1355R	AGC CAG CAA CAT CTT CAA ACT
JCM1722R	AGC TGC TCA TGA TAC GTC TCT AAG
JCM2241R	ACT TGA GGT GTT GAT TCT GTT GG
JCM2665R	GCC ATT TTC GCA TAA GAC AA

natant with a QIAamp Viral RNA kit (Qiagen, Valencia, CA). Group- and virus-specific primers then were used to amplify specific regions of the viral genome with a Titan RT-PCR kit (Roche Diagnostics, Indianapolis, IN). Primers used for RT-PCR included previously published California serogroup/*Bunyavirus* S segment–specific primers BCS82C/BCS332V (251-base pair [bp] product) and JC S segment–specific primers JCS63C/JCS667V (605 bp product) (Kuno et al. 1996). Deoxyribose nucleic acid (DNA) fragments amplified by RT-PCR were sequenced with a ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq DNA Polymerase FS (PE Applied Biosystems, Foster City, CA) and analyzed with a model 377 Prism automated DNA sequencer (PE Applied Biosystems). Nucleic acid sequences were compared to the Genbank database by using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). Further characterization included amplification, sequencing, and phylogenetic analysis of a portion of the M segment of the viral genome. Jamestown Canyon virus M segment–specific primers (Table 1) were designed based on previously published JC M segment sequence (Genbank U88058). Primer pairs JCM7F/JCM845R, JCM821F/JCM1355R, and JCM1093F/JCM2665R were used to amplify by RT-PCR a portion of the M segment that included the G2 and NSM genes and partial G1 gene. These primers, along with the remaining primers listed in Table 1, then were used to sequence the amplified DNA. The resulting sequence was translated into amino acid (aa) sequence and both na and aa sequences were aligned with other *Bunyavirus* sequences by using PILEUP in the Wisconsin Package ver. 10.2 (Devereux et al. 1984). The alignment included 8 members of the CAL serogroup (Inkoo [U88059 and U88060], JC [U88058], Jerry Slough [AF123487], South River [AF123488], Melao [U88057], Serra do Navio [AF123498], KEY [AF123489], and LAC [U18980]), and Oropouche virus (AF312381), a member of the Simba serogroup, was included as an outgroup. Phylogenetic analysis of the aligned sequences by maximum parsimony and neighbor-joining methods was conducted by using MEGA ver. 2.1 (Kumar et al. 2001) and PAUP ver. 4.08b (Swofford 1998).

Parity determination and survival rate: Host-seeking adults were chilled on wet ice immediately after collection in the field, transported to the laboratory, and immobilized in a refrigerator before dissection. The specimens were placed in saline solution (Hayes 1953) and ovaries were removed to evaluate the skeins of the ovarian tracheal system with a compound microscope. Determination of parity was based on the condition of the tracheal system as described by Detinova (1962). The proportion of parous individuals by month was determined by calculating the number of parous individuals divided by the number of nulliparous individuals plus the number of parous individuals. Because gonotrophic cycles vary with temperature throughout the season, survival rate ranges of *Ae. albopictus* were calculated (Davidson 1954) by using estimated gonotrophic cycle lengths of 4.6 days for 26°C and 10 days for 20°C (Hawley 1988). Host-seeking adults not used for parity determination were sorted by species and sex and placed into pools for virus testing.

Weather patterns: Daily precipitation records for the Knox County site were collected from Tennessee Valley Authority (TVA) rainfall gauge 0785 (Bull Run) physically located in Anderson County, Tennessee, but only 6.4 km from the site. Daily precipitation for the Cocke County site was measured by TVA rainfall gauge 0538 (Cosby) physically located in Cosby, Tennessee, but only 7.7 km from the site.

Maximum and minimum daily temperatures of the Knox County site were supplied by the Environmental Science Division of Oak Ridge National Laboratory in Oak Ridge (Anderson County), TN, which is approximately 8.0 km from the Knox County site. The daily temperatures for the Cocke County site were collected by a weather monitoring station located in Gatlinburg (Sevier County), TN, and operated by the U.S. Department of Commerce, National Weather Service, in Morristown, TN, 24 km from the case site.

Statistical analysis: All analyses were performed as described in SAS® (1989). The effects of precipitation (3 wk prior), temperature (3 wk prior), and date on mean number of eggs per trap-week were analyzed separately for each site and species by regression analysis (PROC GLM of SAS). Monthly differences in the proportions of parous individuals at each site were tested by using analysis of variance (ANOVA; PROC GLM of SAS). All residuals were normal (Shaprio–Wilk test for normality) by using PROC UNIVARIATE of SAS and the count data did not require transformation. The seasonal mosquito collections by species between sites were analyzed by using Pearson correlation coefficient of SAS. Chi-square 2 × 2 table analysis was used to determine significant differ-

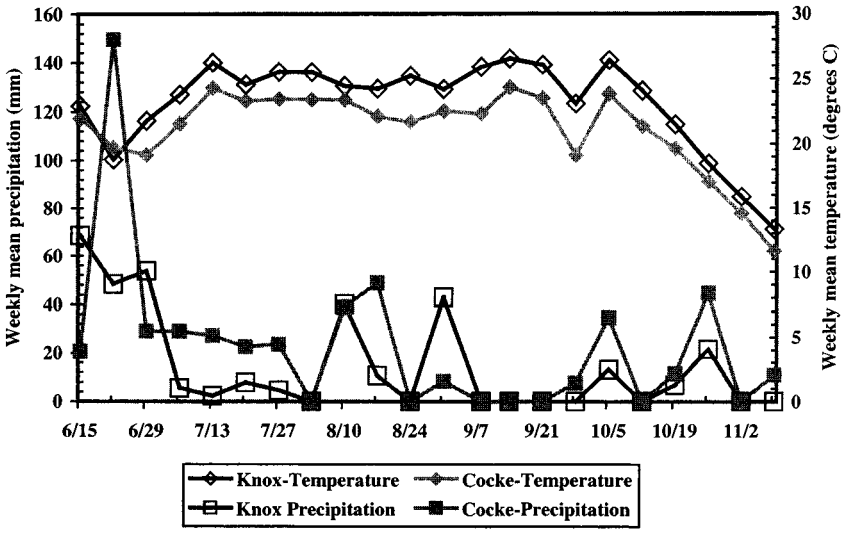

Fig. 1. Precipitation (mm) and temperature (°C) collected from Tennessee Valley Authority rainfall gauges, Oak Ridge National Laboratory, and the National Weather Service in Morristown, TN, from June 15 to November 9, 1998, for the Knox County and Cocke County sites in Tennessee.

ences of oviposition traps positive for each species in each county.

RESULTS

Weather patterns

The weekly means of precipitation from June 15 to November 9 for Knox and Cocke counties are presented in Fig. 1. With the exception of large rainfall documented in June in Cocke County, the weekly rainfall means of both counties remained under 50 mm. Rainfall was recorded in only 1 week between August 24 and September 21 in both counties. The mean weekly temperatures of both sites followed the same seasonal pattern, with Cocke County consistently 2–3°C lower than Knox County. The accumulation of precipitation in potential breeding locations was not evaluated during this study at either LAC homesite. Weather data were analyzed and no significant relationships were found between precipitation or temperature and egg collections for either site.

Temporal patterns in oviposition

Of the 2 container-inhabiting mosquitoes found in our study, *Oc. triseriatus* (n = 126,670) made up 77% of the total eggs and *Ae. albopictus* (n = 38,230) made up 23% (Tables 2 and 3). In Knox County, the ratio of *Oc. triseriatus* to *Ae. albopictus* was 4.8:1 and in Cocke County the ratio was 2.3:1. The mean number of eggs deposited by *Oc. triseriatus* was 87.0 and 59.2 eggs per trap-week in Knox and Cocke counties, respectively. The mean number of *Ae. albopictus* was 18.0 and 26.0 eggs per trap-week in Knox and Cocke counties, respectively. Eggs of *Oc. triseriatus* were consistently collected in larger numbers than eggs of *Ae. albopictus* from both sites. A high correlation was found between the numbers of eggs for both *Oc. triseriatus* (r^2 = 0.67, $P \leq 0.0001$) and *Ae. albopictus* (r^2 = 0.76, $P \leq 0.0001$) between the Knox County and Cocke County sites.

The oviposition activity of each species in June (Cocke County) and June–July (Knox and Cocke counties) (Table 4) was statistically evenly distributed in the ovitraps. As the summer progressed, eggs of *Ae. albopictus* were found in a significantly larger proportion of the ovitraps from both counties.

Rearing success

The rearing success of eggs collected from both counties was higher for *Ae. albopictus* (16.6 and 15.8%) than for *Oc. triseriatus* (1.6 and 1.7%) (Tables 2 and 3) even after 2 hatching attempts. Because of the unexpected low rearing success, 2 sets of 5 random samples of oviposition strips collected were treated with Trpis bleaching solution and the eggs were examined for embryonic development. Embryonic development could not be detected and all of the eggs appeared to be desiccated.

Adults tested for virus

In total, 8,243 mosquitoes (*Ae. albopictus* = 6,158 and *Oc. triseriatus* = 2,085) were reared to the adult stage (281 pools) and 165 host-seeking adult mosquitoes (*Ae. albopictus* = 155, *Oc. triseriatus* = 10) (32 pools) were tested for viruses. One JC subtype isolate (TN98-5085) was made

Table 2. Number of eggs collected and adults reared (male and female) of container-inhabiting mosquitoes by date in Knox County, TN, 1998.

		Ochlerotatus triseriatus			*Aedes albopictus*		
			Adults reared			Adults reared	
Date	No. ovitraps	No. eggs	Male	Female	No. eggs	Male	Female
June 17	40	9,020	140	172	291	79	76
June 22	40	5,090	56	40	686	93	109
June 29	40	5,869	0	0	1,600	69	70
July 6	39	8,806	84	86	1,581	218	198
July 13	40	9,877	11	4	1,131	186	162
July 20	40	10,996	228	366	1,373	198	239
July 27	40	6,082	4	0	1,210	21	21
Aug. 3	40	5,160	18	7	516	117	106
Aug. 10	39	3,230	0	0	283	0	3
Aug. 17	40	2,397	0	0	2,654	130	112
Aug. 24	40	2,315	0	0	1,719	95	109
Aug. 31	39	1,563	0	0	1,013	51	37
Sept. 8	39	1,417	0	0	565	6	4
Sept. 14	40	385	0	0	128	0	0
Sept. 21	39	1,240	0	0	151	0	1
Sept. 28	40	749	0	0	149	5	2
Oct. 5	38	640	0	0	162	32	37
Oct. 12	40	91	0	0	195	0	0
Oct. 19	40	207	0	0	125	0	0
Oct. 26	38	49	0	0	4	0	0
Nov. 2	38	47	0	0	67	0	0
Nov. 9	36	1	0	0	2	0	0
Total	865	75,231	541	675	15,605	1,300	1,286

Table 3. Number of eggs collected and adults reared (male and female) of container-inhabiting mosquitoes by date in Cocke County, TN, 1998.

		Ochlerotatus triseriatus			*Aedes albopictus*		
			Adults reared			Adults reared	
Date	No. ovitraps	No. eggs	Male	Female	No. eggs	Male	Female
June 17	40	4,077	164	187	235	71	76
June 22	40	6,148	38	41	1,058	111	133
June 29	40	6,560	2	1	2,009	261	211
July 6	40	6,498	21	19	1,899	265	267
July 13	39	6,296	72	137	1,084	161	201
July 20	40	3,003	73	112	1,426	217	239
July 27	39	2,155	1	1	1,248	33	31
Aug. 3	40	1,915	0	0	712	45	53
Aug. 10	38	1,340	0	0	1,645	6	6
Aug. 17	40	1,150	0	0	2,581	70	62
Aug. 24	40	1,739	0	0	1,697	115	122
Aug. 31	39	1,776	0	0	1,777	64	46
Sept. 8	40	2,163	0	0	1,650	19	18
Sept. 14	38	1,359	0	0	337	54	47
Sept. 21	40	2,072	0	0	382	55	69
Sept. 28	39	706	0	0	627	87	55
Oct. 5	38	1,107	0	0	670	113	177
Oct. 12	40	382	0	0	606	9	3
Oct. 19	40	424	0	0	574	0	0
Oct. 26	40	114	0	0	73	0	0
Nov. 2	39	437	0	0	253	0	0
Nov. 9	40	18	0	0	82	0	0
Total	869	51,439	371	498	22,625	1,756	1,816

Table 4. Ovitraps positive for *Ochlerotatus triseriatus*, *Aedes albopictus*, or both in eastern Tennessee in 1998.

County	Month	*n*	No. positive (%)[1] *Oc. triseriatus*	*Ae. albopictus*	Both species
Cocke	June	120	85a	80a	68.3
	July	158	74.7a	95.6b	73.4
	August	197	48.2a	90.4b	41.1
	September	157	65.6a	82.8b	56.1
	October	158	41.8a	71.5b	34.8
	November	79	15.2a	41.8b	5.1
Knox	June	120	90a	82.5a	75
	July	159	94.3a	93.1a	86.8
	August	198	68.2a	79.8b	51.5
	September	158	43.7a	68.3b	34.2
	October	156	15.4a	37.8b	10.1
	November	74	4.1a	13.5b	0

[1] Values followed by the same letters are not significantly different ($P > 0.05$) when analyzed by chi-square 2 × 2 table.

Table 5. A 1-way typing plaque-reduction neutralization test (PRNT) that provisionally identifies virus isolate TN98-5085 as a closely related Jamestown Canyon–like virus.[1]

Test virus	Single-injection antibody to	Heterologous/homologous neutralization titers
TN98-5085) (84 PFU)	CE	<40/320
	KEY	<32/256
	JC	≥10,240/10,240
	LAC	320/5,120
	SA	320/5,120
	SSH	640/10,240
	TVT	40/640
	JC MCA	40/2,560

[1] PFU, plaque-forming units; CE, California encephalitis virus; KEY, Keystone virus; JC, Jamestown Canyon virus; LAC, La Crosse virus; SA, San Angelo virus; SSH, snowshoe hare virus; TVT, trivittatus virus; MCA, monoclonal antibody.

from a pool of 50 male *Ae. albopictus* collected in ovipostion traps from Knox County during the week of July 20–27, 1998. The MIR calculated for this period was 1.89 per 1,000 *Ae. albopictus* adults (n = 529).

Virus identification

Preliminary PCR testing indicated that the virus isolated was a CAL serogroup member. The virus isolate was then tested in a 1-way PRNT against single-injection antisera to 7 domestic CAL serogroup viruses and a type-specific neutralizing MCA to JC virus. The PRNT results showed that, with the antisera tested, the TN98-5085 virus isolate was most closely related to JC (Table 5). The JC single-injection antibody neutralized TN98-5085 to equal to or greater than the homologous titer. Single-injection antibody to the 6 other CAL serogroup viruses neutralized the virus isolate 8- to 16-fold less than the homologous titers. The JC MCA neutralized the virus isolate to a titer of 40, whereas the homologous PRNT titer versus prototype JC was ≥1,280.

Deoxyribose nucleic acid fragments were successfully amplified from extracted TN98-5085 viral RNA by RT-PCR with S segment–specific primers. The na sequences of these fragments were compared with the Genbank database to verify the preliminary identification of the virus by PRNT. Basic Local Alignment Search Tool (Altschul et al. 1990) search results with the 251- and 605-bp fragment sequences indicated closest homology to Inkoo virus (97 and 94%, respectively) and JC (96 and 93%, respectively). A larger fragment (2,612 bp) of the M segment of the virus isolate RNA was amplified, sequenced, and translated into aa sequence (Genbank accession AF468197). Phylogenetic analysis conducted on an alignment of the TN98-5085 na and aa sequences with those of other bunyaviruses placed the isolate in a clade that included the CAL serogroup viruses Inkoo virus, JC, and Jerry Slough virus. This group of viruses is genetically very closely related and phylogenetic trees generated by maximum parsimony analysis were unresolved relative to the terminal positions of these taxa.

Parity determination and survival rates

Host-seeking adults of *Ae. albopictus* (n = 567) (Table 6) and *Oc. triseriatus* (n = 25) were dissected to determine parity. Gravid *Ae. albopictus* females collected in Knox County (n = 5) and Cocke County (n = 9) were treated as parous in-

Table 6. Parity status of *Aedes albopictus* in host-seeking populations from Knox and Cocke counties, TN (July 7–October 27, 1998).

Month	Knox County Total dissected	Parous	Nulliparous	Parity rate	Cocke County Total dissected	Parous	Nulliparous	Parity rate
July	72	56	16	77.8	49	39	10	79.6
August	127	101	26	79.5	99	91	8	91.9
September	66	56	10	84.8	67	53	14	79.1
October	42	33	9	78.6	45	40	5	88.9
Total	307	246	61	80.2	260	223	37	85.8

dividuals. The monthly mean proportion of parous *Ae. albopictus* in Knox County from July to October ranged from 0.78 to 0.85; proportions in Cocke County ranged from 0.79 to 0.92. The monthly parity rates did not differ significantly (ANOVA, $P \leq 0.05$) between months at each site. Calculated daily survival rates of female *Ae. albopictus* during the 4-month period ranged from 96% during September (mean temperature 24.9°C, 4.6-day estimated gonotrophic cycle) to 99% during October (mean temperature 15.7°C, 10-day estimated gonotrophic cycle). Although host-seeking adult *Oc. triseriatus* were collected and dissected, the numbers of individuals (Knox County $n = 13$, Cocke County $n = 12$) were too low for comparison to *Ae. albopictus*. The proportion of parous adults for *Oc. triseriatus* were 0.71 and 0.80 for Knox and Cocke counties, respectively.

Other species of mosquitoes were collected in host-seeking traps in addition to *Oc. triseriatus* and *Ae. albopictus*. Knox County collections included *Aedes vexans* (Meigen), *Ochlerotatus trivittatus* (Coq.), *Oc. canadensis* (Theobald), *Oc. sticticus* (Meigen), *Culex pipiens* L., *Cx. erraticus* (Dyar and Knab), *Psorophora horrida* (Dyar and Knab), *Ps. ferox* (Von Humboldt), and *Anopheles punctipennis* (Say). Host-seeking adults collected in Cocke County included *Ae. vexans, Oc. trivittatus, Oc. sticticus, Oc. mitchellae* (Dyar), *Cx. pipiens, Cx. erraticus, Ps. ferox, An. punctipennis,* and *An. quadrimaculatus* Say.

DISCUSSION

Ochlerotatus triseriatus was more abundant than *Ae. albopictus* in oviposition traps in the vicinity of La Crosse viral encephalitis case sites in eastern Tennessee, whereas *Ae. albopictus* was more abundant in traps sampling host-seeking adults. This phenomenon also was observed at other collection sites in eastern Tennessee (Gottfried 1999), suggesting that populations of *Oc. triseriatus* are undersampled by CO_2-baited traps compared to populations of *Ae. albopictus*. However, *Oc. triseriatus* also possibly experiences higher larval mortality than does *Ae. albopictus*.

The temporal distributions of *Ae. albopictus* and *Oc. triseriatus* from Knox and Cocke counties were significantly correlated, although the 2 sites are in different forest types and are located 82 km apart. The similarities of the temporal patterns of each species at the 2 sites suggest that the populations fluctuate in similar manners throughout the region, regardless of numerous local factors that impact those populations. This could be an important factor in regional surveillance of container-inhabiting mosquitoes because surveillance sites could be selected based on logistical (personnel convenience and monetary concerns) needs rather than location. The lack of a significant relationship between rainfall, temperature, and number of eggs collected was not surprising, given the multitude of other ecological and biological factors that may influence population density.

The high numbers of eggs of *Oc. triseriatus* collected on the 1st collection date in June indicated that the onset of adult feeding and oviposition activity began considerably before this date. *Aedes albopictus* collections were lower on the 1st collection date, indicating either that oviposition activity began later than for *Oc. triseriatus* or that the population of this species build at a slower rate over the season. In a concurrent study at another site in Knox County (Gottfried 1999), oviposition traps were placed in the field during the 1st week in March. Eggs of *Oc. triseriatus* were 1st collected between April 28 and May 11, 1998, and between May 3 and 10, 1999. Similar results were found in 1999, when eggs of *Ae. albopictus* were 1st collected between May 19 and 25, 1998, and between May 11 and 17, 1999 (Gottfried 1999).

The overall rearing success was lower than expected for *Oc. triseriatus* and *Ae. albopictus*. Studies of LC conducted in West Virginia in 1996 yielded 23% rearing success of *Oc. triseriatus* (R. Nasci, unpublished data). Rearing failure caused by natural egg death and oviposition of diapausing eggs is a common phenomenon; however, these 2 facts do not entirely explain the low rearing success and hatch rate. The ovipostion strips were transported to the laboratory in sealed plastic bags not protected from the heat and were stored in darkness for approximately 3 wk before rearing attempts. The heat produced in the sealed plastic bags may have killed the eggs or improper storage procedures may have prevented the eggs from completing embryonation. Oviposition strips should have been protected from adverse conditions during transportation to the laboratory and placed under long day length conditions (14:10 h light:dark) and 27°C for 1 wk to assure that the eggs were embryonated (Szumlas et al. 1996a).

Aedes albopictus was the only mosquito that was collected in sufficient numbers to evaluate parity over the 4-month period. The monthly proportion of parous females was high compared to parity rates observed in Louisiana (Willis and Nasci 1994). The estimated survival rate for adult female *Ae. albopictus* (0.92–0.99) exceeded the survival rate (0.87–0.89) reported in a mark and recapture study conducted in a Missouri scrap tire yard (Niebylski and Craig 1994). Factors that were not investigated in this study, such as body size (wing lengths), nutritional quality of larval habitats, proximity of blood-meal hosts, and migration of individuals may influence parity rates and may have accounted for the observed differences in the proportion that were parous. The high parity and survival rates indicate that the population was multiparous. This leads to the conclusion that a large percentage of the individuals have the potential to contact multiple hosts over their lifespan, and have

greater potential for ingesting a pathogen, surviving the extrinsic incubation period, and ultimately transmitting the pathogen to a new host (Haramis 1983, Paulson and Hawley 1991).

The results of the PRNT and genetic analyses, along with the geographic location from which the virus was isolated, suggest a provisional identification of the TN98-5085 virus isolate as a CAL serogroup virus, most likely a subtype of JC. Previous research has demonstrated the close genetic relationship that exists between the Inkoo virus and JC, and Jerry Slough virus is considered a variant of JC (Karabatsos 1985, Campbell and Huang 1999). Further investigation is being conducted to clarify the antigenic and genetic relationships among these viruses to facilitate future identification of virus isolates.

Nevertheless, this is the 1st report of isolation of a JC-like virus from *Ae. albopictus* and the 1st evidence of transovarial transmission of this human pathogen from a naturally occurring population of *Ae. albopictus*. Additionally, this is the 1st time that a JC-like virus has been isolated from mosquitoes in Tennessee, further verifying the enzootic prevalence of the virus in temperate North America (Grimstad 1988).

Human disease caused by JC is reportable in Tennessee, although cases have never been reported to the Tennessee Department of Health (T. Jones, personal communication). Geographically, the nearest human seroepidemiology study was conducted in the Cherokee Indian Reservation, North Carolina. Antibody to JC was detected but was relatively rare, indicating that the virus is transmitted infrequently to humans on the reservation (Szumlas et al. 1996b).

In 1997, 10 pediatric cases of La Crosse viral encephalitis were reported to the Tennessee Department of Health (T. Jones, personal communication). Seven of the 10 cases were confirmed by plaque-reduction neutralization on serum samples. The remaining 3 cases were diagnosed based on a 4-fold rise in immunofluorescent antibody titers. In 1998, serum from children in Tennessee with clinical signs consistent with arboviral encephalitis were tested for eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, and CAL serogroup antibodies by using the immunofluorescence antibody assay. Based on serologic, clinical, and epidemiologic information, positive results in 1998 were assumed to indicate LAC infection. Antibody cross-reactivity between the CAL serogroup viruses commonly occurs and it is possible that some persons with evidence of antibodies to CAL serogroup viruses may have been exposed to JC. However, Calisher (1994) reported that the only confirmed JC infection reported in a person <18 years of age was an 8-year-old child that had another similar virus, making the diagnosis suspect.

Grimstad et al. (1982) presented preliminary evidence that clinical cases of CAL serogroup viruses were not being confirmed serologically. Because JC has the broadest spectrum of clinical symptoms of the CAL serogroup members (Grimstad 1988), many CAL serogroup virus infections possibly go undiagnosed. The isolation of a JC-like virus from *Ae. albopictus* suggests that the virus may be the cause of undiagnosed encephalitis cases as well as inapparent mild clinical influenzalike sickness in the human population (Grimstad 1988).

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